JPP Journal of Pharmacy and Pharmacology

Royal Pharmaceutical Society of Great Britain

JPP Journal of Pharmacy and Pharmacology

Research Paper

JPP 2010, 62: 1003–1009

Received August 20, 2009
Accepted April 15, 2010
DOI 10.1111/j.2042-7158.2010.01118.x
ISSN 0022-3573

Quercetin and naringenin reduce abnormal development of mouse embryos produced by hydroxyurea

Ricardo Pérez-Pastén, Elizdath Martínez-Galero and Germán Chamorro-Cevallos

Departamento de Pharmacia, Escuela Nacional de Ciencias Biológicas, I.P.N. Campus Zacatenco, México D.F., México

Abstract

Objectives There is limited evidence about the impact of quercetin and naringenin on embryonic development. The purpose of this work was to evaluate *in vitro* their direct teratogenic potential as well as their protective activity against teratogenesis mediated by oxidative damage on mouse embryos.

Methods Quercetin and naringenin toxicity on whole mouse cultured embryos, as well as their ability to protect embryos against hydroxyurea-induced insult were evaluated.

Key findings Quercetin 100 μM and naringenin 300 μM produced significant reduction of developmental and growth parameters, in comparison with those of the control group. Embryos exposed to the concurrent administration of quercetin or naringenin with hydroxyurea (2 μM, 2 h) were significantly protected from growth and developmental retardation, and abnormalities induced by hydroxyurea. Interestingly, embryos exposed to hydroxyurea and dimethyl sulfoxide 0.1%, the vehicle employed to dissolve flavonoids, also showed significant damage amelioration.

Conclusions These results indicate that quercetin and naringenin have not only a minor toxic effect on development, but also a protective effect against hydroxyurea-induced embryonic damage.

Keywords flavonoids; hydroxyurea; teratogenesis; whole embryo culture

Introduction

The chemoprotective activity of flavonoids has attracted attention in the last few years because it has been shown that these compounds exhibit a broad variety of pharmacological properties, such as anti-tumour, anti-platelet, anti-ischaemic, antiviral, anti-ulcer and anti-inflammatory, as well as cataract protective effects.[1] These biological effects have been attributed to their antioxidant activity with a strong free radical scavenging capacity.[2]

Naringenin has been identified as an antioxidant, anti-cancer, and potent cholesterol-lowering agent in previous animal studies.[3] Due to its antioxidant properties, it also suppresses cytotoxicity and apoptosis in mouse leukaemia P388 cells exposed to hydrogen peroxide,[4] or to the anti-cancer drug cytosine arabinoside.[5] Similarly, it has been shown that quercetin scavenges free radicals directly, inhibits xanthine oxidase and lipid peroxidation,[6,7] alters the antioxidant defence pathway *in vivo* and *in vitro*, suppresses cell proliferation, protects against LDL oxidation, prevents platelet aggregation and induces apoptosis.[8,9]

On the other hand, oxidative damage is not only a critical mechanism in the development of several diseases, including HIV infection, neurodegeneration, diabetes, angina and certain forms of cancer,[10] but also has an important role in the aetiology of several congenital anomalies. There is evidence that some teratogens may affect developing embryos by increasing the production of reactive oxygen species, which, in combination with the particular weak antioxidant defence in the early stages, results in embryonic damage.[11] The connection between oxidative damage and teratogenesis has been documented for maternal diabetes, radiation, cocaine, ethanol, phenytoin and thalidomide, among others.[12]

Hydroxyurea is a useful teratogen employed to understand the association between developmental toxicity and oxidative stress.[13,14] It acts as an inhibitor of ribonucleotide reductase, an enzyme responsible for deoxyribonucleotides synthesis,[15] and as inductor of oxidative stress by generating free radicals.[16] Hydroxyurea exposure induces extensive cell

Correspondence: Professor Germán Chamorro-Cevallos, Escuela Nacional de Ciencias Biológicas, I.P.N. Campus Zacatenco, Ave Wilfrido Massieu S/N, 07738, México D.F., México.
E-mail: gchamcev@yahoo.com.mx

death in the neural tube region and limb buds.[17–19] It has been shown to produce growth retardation, neural tube and facial defects, as well as limb defects in post-implanted cultured embryos.[16]

The purpose of this work was to evaluate *in vitro* the direct teratogenic potential of quercetin and naringenin, as well as their protective activity against teratogenesis mediated by oxidative damage in mouse embryos.

Materials and Methods

Animals

Experimental procedures were reviewed and approved by our Institutional Committee in accordance with the ethical principles and regulations as specified by the National Institute of Health of Mexico official regulation 'Norma Oficial Mexicana para el cuidado y manejo de animales de laboratorio NOM-ZOO-062-1999'.

Male and female CD1/ICR mice, 25–30 g, from the Birmex S.A. (Mexico City, Mexico) were used. The mice were kept at room temperature (24 ± 2°C) in a 12-h light–dark cycle, light on at 0800 h, with free access to tap water and commercial pellet diet. Mating was carried out by placing three females with one male from 0600 h to 0800 h. Gestational day (GD) 0 was designed with the confirmation of pregnancy by the detection of vaginal sperm plug.

Whole embryo culture

On GD 8 at 0900 h, pregnant mice were killed by cervical dislocation and the whole uteri were removed. Conceptuses were prepared according to the Cockroft technique.[20] Briefly, each conceptus was dissected from the uterus and decidual tissue in Hank's buffered saline solution (HBSS) (Gibco Co.). Reichert's membrane and parietal yolk sac were removed, leaving the visceral yolk sac and the ectoplacental cone intact. Only five to four somites embryos were used for culture and randomized to one of each group: group 1, rat serum control; group 2, culture media with DMSO 0.1% (Sigma Chemical Co); groups 3–6, quercetin or naringenin (Sigma Chemical Co) at final concentrations of 10, 30, 100 and 300 μg/ml respectively, diluted with DMSO 0.1% in culture medium. As many as four embryos were transferred to 60-ml sterile glass bottles containing a final volume of 4 ml sterile heat-inactivated rat serum at 37°C added with or without test compounds (prepared following the Cockroft[20] indications). Culture bottles were placed in a roller incubator (BTC Engineering, Milton, Cambridge, UK) at 37°C and 60 rev/min. Each vial was gassed before and immediately after the embryo transfer, using a mixture of 5% O_2, 5% CO_2 and 90% N_2. The gas mixture was changed as follows: at 15 h, 15% O_2, 5% CO_2; at 24 h, 45% O_2, 5% CO_2; at 36 h, 90% O_2, 5% CO_2. In all cases the mix was N_2 balanced.

All embryos were removed after 48 h, placed in HBSS at 37°C, and examined for viability and development by using a dissecting microscope. Viability was assessed by the presence of yolk sac circulation and heartbeat. Only live embryos were considered for further analysis. Yolk sac circulation was evaluated while diameters were measured and first membrane and amnion were removed. Embryonic crown–rump and head lengths were measured; embryos were examined for number of somites and rated by a morphological score described elsewhere.[21] Abnormal embryos (having at least one abnormality) were classified according to location and features. All embryos alive (normal or defective) were placed in cryogenic tubes with 900 μl of DNA assay buffer (10 mM $NaH_2PO_4 \cdot 5H_2O$, 40 mM Na_2HPO_4 anhydrous, 2 M NaCl, 2 mM disodium EDTA, pH 7.4 (J.T. Baker)) and kept at −70°C for later analysis.

The interaction between hydroxyurea and naringenin or quercetin was performed according to the method described above, but with the following modifications: embryos were transferred to 60-ml sterile glass bottles containing a final volume of 4 ml culture medium at 37°C added with hydroxyurea 2 mM (Sigma Chemical Co) with or without each flavonoid at final concentrations of 3 or 30 μM. Culture bottles were placed in a roller incubator. Each vial was gassed before and immediately after the embryo transfer, using a mixture of 5% O_2, 5% CO_2 and 90% N_2. Immediately after 2 h of incubation, treated embryos were washed with HBSS and transferred to new bottles containing fresh culture medium free of chemicals. Each vial was against gassed using the same gas mixture and was replaced in the roller incubator to continue the culture for the remaining time up to 48 h according to the aforementioned procedures.

DNA quantification

In accordance with the method of Labarca and Paigen,[22] embryos were thawed and diluted with DNA assay buffer to a final volume of 1.9 ml and then sonicated (15 s, 4 μm) by using a Soniprep (Gallenkamp). Each sample was poured into glass cuvette and 100 μM of 20 μg/ml Hoechst 33258 (Sigma Chemical Co) solution was added immediately. The mixture was stirred and the relative fluorescence (RF) at 460 nm emission and 365 nm excitation was read on a spectrofluorometer (Perkin-Elmer LS45). The samples were read five consecutive times, being mixed between each reading. DNA content was determined by reference to a standard line of calf thymus DNA (Sigma Chemical Co) and expressed as μg DNA/embryo.

Statistics

Differences between means were evaluated by one-way analysis of variance followed by Newman–Keuls multiple comparison test. Intergroup comparison of malformation incidence was performed with Fisher's exact test at the 95% significance level or chi square (with Yate's correction), whichever was applicable. Data were analysed using the Sigma Stat software Ver. 2.07, and presented as means ± standard error. $P < 0.05$ was considered significant.

Results

Effect of naringenin and quercetin on in-vitro mouse embryo development

Several concentrations of naringenin and quercetin were tested in separate assays to determine their effects on embryonic development. Table 1 shows that although naringenin at 100 μM increased the total incidence of abnormal embryos, only at 300 μM did it produce a significant increase of abnor-

Table 1 Effect of naringenin on cultured mouse embryos

	Normal	Naringenin (μM)				DMSO
		10	30	100	300	
Total embryos	24	24	24	24	24	24
Viable embryos	24	24	24	24	22	17[a]
DNA (μg/embryo)	29.3 ± 0.17	29.4 ± 0.12	29.5 ± 0.16	29.5 ± 0.22	29.2 ± 0.18	24.5 ± 0.42[a]
Vitelin sac diameter (mm)	4.3 ± 0.90	4.1 ± 0.11	3.9 ± 0.33	3.9 ± 0.12	3.2 ± 0.33	3.2 ± 0.56
Head–rump length (mm)	4.2 ± 0.97	4.2 ± 0.13	4.0 ± 0.31	3.8 ± 0.41	3.7 ± 0.14	3.3 ± 0.18[a]
Cephalic length (mm)	2.4 ± 0.75	1.9 ± 0.19	2.1 ± 0.16	1.9 ± 0.23	1.9 ± 0.46	1.7 ± 0.25
No. of somites	30.4 ± 1.20	29.0 ± 0.5	29.3 ± 1.11	30.3 ± 1.35	26.0 ± 1.72	25.0 ± 0.98[a]
Morphological score	42.1 ± 1.5	43.3 ± 1.76	40.8 ± 1.67	41.3 ± 2.01	39.3 ± 1.97	32.6 ± 1.66[a]
Abnormal (*n*)	0	2	1	1	6[a,b]	9[a,b]
Neural tube defects (*n*)	0	0	0	0	3	4
Oedema (*n*)	0	1	1	1	4	6[a,b]
Rotation failure (*n*)	0	1	0	0	3	8[a,b]
Telencephalic hypoplasia (*n*)	0	0	0	0	2	9[a,b]

Mean ± SEM. [a]$P < 0.05$ vs normal. [b]$P < 0.05$ vs control (DMSO 0.1%). *n*, Number of defective embryos from total alive. Incidences were evaluated by χ^2 with Yates's correction or Fisher's Exact Test; means by analysis of variance, Newman–Keuls when applicable.

Table 2 Effects of quercetin on cultured mouse embryos

	Normal	Quercetin (μM)				DMSO
		10	30	100	300	
Total embryos	24	24	24	24	24	24
Viable embryos	24	24	24	24	18[a]	14[a]
DNA (μg/embryo)	29.7 ± 0.15	29.8 ± 0.18	29.1 ± 0.20	29.4 ± 0.17	25.6 ± 0.11[a]	22.3 ± 0.14[a]
Vitelin sac diameter (mm)	4.3 ± 0.90	4.1 ± 0.11	4.1 ± 0.13	3.7 ± 0.29	3.2 ± 0.33	3.0 ± 0.43
Head–rump length (mm)	4.2 ± 0.37	4.2 ± 0.13	4.0 ± 0.20	4.3 ± 0.27	3.1 ± 0.16[a]	2.8 ± 0.18[a]
Cephalic length (mm)	2.4 ± 0.75	1.9 ± 0.09	2.3 ± 0.15	1.9 ± 0.13	1.1 ± 0.26[a]	1.1 ± 0.15[a]
No. of somites	30.4 ± 1.20	29.0 ± 0.5	28.3 ± 1.11	28.6 ± 1.30	26.0 ± 1.72	25.0 ± 0.98[a]
Morphological score	49.1 ± 1.5	48.3 ± 1.76	47.8 ± 1.65	47.6 ± 2.82	39.3 ± 1.97[a]	38.6 ± 1.66[a]
Abnormal (*n*)	0	2	1	1	8[a,b]	13[a,b]
Neural tube defects (*n*)	0	0	0	0	4	8[a,b]
Oedema (*n*)	0	1	1	0	6[a,b]	9[a,b]
Rotation failure (*n*)	0	1	0	1	5[a,b]	9[a,b]
Telencephalic hypoplasia (*n*)	0	0	0	0	4	10[a,b]

Mean ± SEM. [a]$P < 0.05$ vs normal. [b]$P < 0.05$ vs control (DMSO 0.1 %). *n*, Number of defective embryos from total alive. Incidences were evaluated by χ^2 with Yates's correction or Fisher's Exact Test; means by analysis of variance, Newman–Keuls when applicable.

malities such as telencephalic hypoplasia, rotation failure and oedema, as well as viability decrease and growth retardation.

On the other hand, the embryolethal, embryotoxic and teratogenic concentration of quercetin were 3-fold lower than those of naringenin (Table 2). At 100 μM quercetin caused a significant increase in the incidence of oedema and rotation failure, while at 300 μM neural tube defects, somite dysmorphology and telencephalic hypoplasia were additionally produced.

Effect of flavonoids on hydroxyurea-induced dysmorphology of cultured mouse embryos

Working concentrations of naringenin and quercetin were 30 μM maximum because in both cases that concentration was the highest having non-teratogenic effects. Embryo exposure to hydroxyurea 2 μM for 2 h (Table 3) led to a significant increase in the incidence of lethality, growth retardation and malformations. The main abnormalities were failure of closure of the neural tube, reduced telencephalic sphere, oedema, rotation failure and erratic somite disposition.

Embryos subjected to co-treatment with hydroxyurea and naringenin (Table 3) showed an improvement in viability, growth and differentiation. Besides, naringenin diminished the frequency of malformations in an apparent dose-dependent fashion, since the lowest incidence of abnormal embryos was observed at 30 μM. Specific defects like neural closure tube failure and abnormal somites were reduced at both naringenin concentrations. With regard to development, growth and malformations, a better protection was obtained when embryos where co-treated with quercetin 3 μM. Moreover, at this concentration the amelioration of hydroxyurea-induced injury concerning these parameters was such that values reached those of the DMSO group. However, when the concentration of quercetin was increased to 30 μM the frequency of live embryos and the number of somites were

Table 3 Effect of naringenin and quercetin on embryonic growth and development of embryos exposed to hydroxyurea

	DMSO	Hydroxyurea	Hydroxyurea				
			DMSO	Naringenin 3 μM	Naringenin 30 μM	Quercetin 3 μM	Quercetin 30 μM
Total embryos	24	28	24	24	24	24	24
Viable embryos	24	18[a]	23[b]	19[a,c]	22[b]	22[b]	17[a,c]
DNA (μg/embryo)	29.2 ± 0.20	24.3 ± 0.31[a]	26.8 ± 0.26[a,b]	27.4 ± 0.29[a,b,c]	28.1 ± 0.28[a,b,c]	28.9 ± 0.25[b,c]	27.9 ± 0.19[a,b,c]
Vitelin sac diameter (mm)	4.4 ± 0.10	3.1 ± 0.14[a]	3.5 ± 0.14[a,b]	3.8 ± 0.20[a,b]	3.8 ± 0.11[a,b]	4.2 ± 0.10[b,c]	3.5 ± 0.15[a,b]
Head–rump length (mm)	4.1 ± 0.13	2.8 ± 0.2[a]	3.0 ± 0.43[a]	3.3 ± 0.06[a]	3.6 ± 0.16[a,b,c]	3.7 ± 0.99[b,c]	3.4 ± 0.18[a,b]
Cephalic length (mm)	1.9 ± 0.09	1.3 ± 0.11[a]	1.8 ± 0.14[b]	1.6 ± 0.12[b]	1.7 ± 0.10[b]	1.8 ± 0.08[b]	1.2 ± 0.12[a,c]
No. of somites	29.4 ± 0.50	22.6 ± 1.55[a]	25.32 ± 1.02[a,b]	26.5 ± 0.35[b]	28.6 ± 0.41[b,c]	26.7 ± 0.84[b]	25.4 ± 0.98[a]
Morphological score	35.4 ± 1.26	24. ± 1.35[a]	30.8 ± 1.20[a,b]	29.9 ± 1.12[a,b]	32.1 ± 0.97[b]	32.9 ± 0.73[b]	27.5 ± 1.45[a,b]
Abnormal (n)	2	18[a]	12[a,b]	11[a,b]	6[a,b]	6[a,b]	10[a,b]
Neural tube defects (n)	0	12[a]	3[b]	0[b]	1[b]	0[b]	5[b]
Abnormal somites (n)	0	8[a]	3[b]	4[a]	3[a,b]	0[b]	5[b]
Oedema (n)	1	14[a]	6[a,b]	8[a,b]	2[b]	4[b]	5[b]
Rotation failure (n)	1	12[a]	6[a,b]	8[a]	0[b,c]	0[b,c]	0[b,c]
Telencephalic hypoplasia (n)	0	14[a]	6[a,b]	9[a]	1[b]	8[a,b]	10[a]

Hydroxyurea treatment: 2 mM for 2 h. Values are mean ± SEM. [a]$P < 0.05$ vs DMSO alone. [b]$P < 0.05$ vs hydroxyurea alone. [c]$P < 0.05$ vs hydroxyurea–DMSO 0.1%. n, Number of defective embryos from total alive. Incidences were evaluated by χ^2 with Yates's correction or Fisher's Exact Test; means by analysis of variance, Newman–Keuls when applicable.

similar to those of hydroxyurea-treated embryos. Meanwhile, the protection on growth and development insult with that concentration decreased and the number of abnormal embryos remained steady.

Additionally, an interesting finding was that embryos exposed to hydroxyurea and DMSO showed a significant decrease in the embryotoxic, embryolethal and teratogenic effects exerted by the former, indicating that DMSO, the vehicle used to dissolve the flavonoids, has itself a protective activity against the toxic effects of hydroxyurea on embryos.

Discussion

The beneficial functions of dietary flavonoids have been well documented, and their potential toxic impact has also been understood progressively. Because flavonoids are used as dietary supplements for health maintenance, it is likely that large amounts may be ingested. The rationale for performing this study was to establish the direct teratogenic activity of quercetin and naringenin during embryonic development, and to determine whether both flavonoids are capable of protecting against teratogenic insult, mediated mainly by oxidative damage.

In this study it was demonstrated that quercetin and naringenin exposure produced growth retardation, developmental defects and reduced viability in cultured mouse embryos. Both flavonoids induced the same malformation patterns, like neural closure tube defects, telencephalic hypoplasia, abnormal somites and rotation failure. Quercetin showed more teratogenic potential since its toxicity was evident starting from 100 μM. The exact mechanism by which quercetin and naringenin produce *in vitro* teratogenesis is yet to be understood. However, it has been suggested that flavonoids may act at a cellular level as pro-oxidants, enzyme inhibitors and mutagens.[23] A proposed cytotoxic mechanism for flavonoids is their pro-oxidant effect, which could be a consequence of polyphenol autoxidation (hydrogen peroxide production) in growth medium or activation by intracellular cytochromes.[24] Other possible mechanisms are inhibition of topoisomerases,[25] proteosome synthesis,[26] or fatty-acid synthesis,[27,28] cell cycle arrest, p53 accumulation and enhanced c-fos and c-myc.[29] On the other hand, structure–activity relationship studies have demonstrated that quercetin is more toxic than naringenin due to the following characteristics: high lipophilicity, the presence of a C2–C3 double bond and orthohydroxylation on the B ring within its structure, redox potential and cell incorporation rate.[30–32] These findings suggest that the higher teratogenicity observed for quercetin in comparsion with naringenin in this study is probably mediated by their reported differences in structure–activity properties. However, more research is necessary to clarify this issue.

There have been no reports concerning the concentration level of naringenin or quercetin in embryos or fetuses after the dam exposure, or whether they have the ability to cross the placental barrier. In this sense, only few flavonoids have been studied, such as the synthetic flavonoid (3-methyl-48,6-dihydroxy-38, 58-diiodoflavone),[33] α-naphthoflavone,[34] catechin[35] and epigallactocatechin gallate and neohesperidin dihydrochalcone, the placental-barrier-crossing capacity of which has been quantified, but no teratogenic potential has

been evaluated.[36] On the contrary placental and fetal tissue levels of genistein[37] and daidzein,[38] which are teratogenic flavonoids because of their phytoestrogenic activity, have been measured, but their toxic effect depends on oestrogen receptor occupancy in the placenta rather than on their ability to cross the placenta or their conceptus concentration level. From this background and the data reported here, the complexity of flavonoids' teratogenic potential can be visualized. Teratogenic response is often linked to teratogen maternal plasma concentration.[39] Peak plasma concentrations of flavonoids depend on the nature of the specific flavonoid, as well as its source, dose, frequency of exposure and the specie. In data published by Felgines *et al*.,[40] naringenin plasma concentrations recorded 10 h after feeding rats with naringenin and other analogues varied from 128 to 144 μM. In our study we found naringenin in vitro embryotoxic activity at a concentration of 300 μM, which is 2- or 3-fold greater than can be achieved by feeding rats with rich flavonoid diets, suggesting that the flavonoid potential to impact embryonic development *in vivo* is remote.

The quercetin teratogenic concentration determined *in vitro* in this study (100 μM) has been achieved experimentally in rats by others, where the plasma concentration reached a value of 115 μM after an oral administration of 1 g daily.[41] Whereas human quercetin plasma concentrations are usually in the low nanomolar range, upon quercetin supplementation they may increase to the high nanomolar or low micromolar range.[42,43] Although quercetin attains plasma concentrations *in vivo* within the range of teratogenic activity, it has been reported not to be teratogenic in rats. Briefly, Willhite[44] proved that up to 2 g/kg of quercetin administered to rats on day 9 of pregnancy or daily from day 6 to 15 of pregnancy produced no evidence of teratogenicity apart from offspring weight reduction. This data implies that the lack of teratogenicity of quercetin observed *in vivo* may be due to maternal–embryonic relationships, such as maternal metabolism, placental transport or embryonic quercetin uptake: factors that alone or combined could limit quercetin bioavailability.

The results of this work suggest that the human teratogenic potential of these flavonoids could be low, because human plasma levels of flavonoids after a flavonoid-rich diet are in a range of 10–500 nM (about 50 nM and 112 nM, for quercetin and nargingenin, respectively),[45] which is about 2000 times lower than the concentration associated with the in-vitro teratogenic response reported here. Nonetheless, further research is needed to definitively demonstrate the safety of consumption of these flavonoids during pregnancy.

On the other hand, there is extensive interest in the progress of strategies to protect embryonic development from insults during organogenesis. Although maternal dietary antioxidant supplementation has clearly been successful in improving fetal outcomes in animal models, such as experimental diabetes,[46] or after exposure to specific teratogens, including hydroxyurea,[17] there is no evidence of the impact of flavonoids against chemical-induced teratogenesis.

It has been reported that mouse embryos exposed to hydroxyurea exhibit growth retardation, high frequency of lethality, neural tube closure defects, rotation failure and abnormal facial processes.[47] The results of this work are consistent with those reported. In this context, our study shows that naringenin and quercetin decrease growth and differentiation affectation caused by hydroxyurea in cultured mouse embryos. However, a total protection by these compounds was not achieved. The in-vivo and in-vitro teratogenicity of hydroxyurea is sensitive to addition of deoxycytidine monophosphate[48] and to antioxidant agents,[49–51] which partially reduced total and specific frequencies of abnormalities.

Dimethyl sulfoxide, which was used as a vehicle for the flavonoids at a concentration 0.1%, protected embryonic development against hydroxyurea-induced damage, contributing to the protection exerted by flavonoids. Recently it has been reported that DMSO has cyto- and embryoprotective[52,53] activity mediated mainly by antioxidant mechanisms, by reducing the production of reactive oxygen.[54] It is likely that DMSO acts by this mechanism against the teratogenic effect of hydroxyurea.

The protection exerted by naringenin and quercetin, as well as DMSO, in this work, is in agreement with that found for other antioxidants regarding hydroxyurea-induced teratogenesis (i.e. mannitol, propylgallate and Trolox-C). In all cases the teratogenic effect of hydroxyurea had not been completely reverted.

Because antioxidant and free radical scavenging activity are documented for naringenin,[55] quercetin[56] and DMSO,[57] it is possible that their protective effect found in this work might be mediated by the same mechanisms. If dietary quercetin and naringenin could provide protection experimentally in in-vivo models, it might be possible that they also provide protection against human teratogenesis mediated by oxidative chemicals.

Conclusions

Teratogenic concentrations of naringenin and quercetin on post-implantation whole mouse cultured embryos were identified, which in conjunction with flavonoid plasma concentrations available in the literature suggests a remote developmental toxic potential. Additionally, the capacity of these agents to partially protect embryonic development from damage induced by hydroxyurea was proved.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by the Secretaría de Investigación y Posgrado – IPN. Authors are fellows of COFAA, EDI and SNI – CONACyT.

References

1. Anjaneluyu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clin Exp Pharmacol Physiol* 2004; 1: 244–248.
2. Tripoli E *et al*. Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. *Food Chem* 2007; 104: 466–479.

3. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* 2002; 22: 19–34.
4. Kanno S *et al*. Effects of naringin on hydrogen peroxide induced cytotoxicity and apoptosis in P388 cells. *J Pharmacol Sci* 2003; 92: 66–70.
5. Kanno S *et al*. Effects of naringin on cytosine arabinoside (Arac)-induced apoptosis in P388 cells. *Life Sci* 2004; 75: 353–365.
6. Plumb W *et al*. Antioxidant properties of flavonol glycosides from green beans. *Redox Rep* 1999; 4: 123–127.
7. Fiorani M *et al*. Quercetin prevents glutathione depletion induced by dehydroascorbic acid in rabbit red blood cells. *Free Radic Res* 2001; 34: 639–648.
8. Arai Y *et al*. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 2000; 130: 2243–2250.
9. Chowa J *et al*. Quercetin, but not rutin and quercitrin, prevention of H_2O_2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. *Biochem Pharmacol* 2005; 69: 1839–1851.
10. Kampa M *et al*. Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. *Breast Cancer Res* 2004; 6: R63–R74.
11. Wells PG *et al*. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicol Appl Pharmacol* 2005; 207: 354–366.
12. Kovacic P, Somanathan R. Mechanism of teratogenesis: electron transfer, reactive oxygen species, and antioxidants. *Birth Def Res C* 2007; 78: 308–325.
13. DeSesso JM. Cell death and free radicals: a mechanism for hydroxyurea teratogenesis. *Med Hypotheses* 1979; 5: 937–951.
14. Yan J, Hales B. Depletion of glutathione induces 4-hydroxynonenal protein adducts and hdroxyurea teratogenicity in the organogenesis stage mouse embryo. *J Pharmacol Exp Ther* 2006; 319: 613–621.
15. Scott W *et al*. DNA synthesis inhibition and cell death associated with hydroxyurea teratogenesis in rat embryos. *Develop Biol* 1971; 26: 306–315.
16. DeSesso JM *et al*. D-mannitol, a specific hydroxyl free radical scavenger, reduces the developmental toxicity of hydroxyurea in rabbits. *Teratology* 1994; 49: 249–259.
17. DeSesso JM. Amelioration of teratogenesis. I. Modification of hydroxyurea-induced teratogenesis by the antioxidant propyl gallate. *Teratology* 1981; 24: 19–35.
18. Warner A *et al*. A comparsion of the in vivo and in vitro response of mammalian embryos to teratogenic insult. *Toxicology* 1983; 28: 271–282.
19. Zucker RM *et al*. Apoptosis and morphology in mouse embryos by confocal laser scanning microscopy. *Methods* 1999; 18: 473–480.
20. Cockroft DL. Dissection and culture of post-implantation embryos. In: Copp AJ, Cockroft DL, eds. *Postimplantation Mammalian Embryos. A Practical Approach*. Oxford: IRL Press, 1990: 15–40.
21. Brown N, Fabro S. Quantitation of rat embryonic development *in vitro*: a morphological scoring system. *Teratology* 1981; 24: 65–78.
22. Labarca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. *Ann Biochem* 1980; 102: 344–352.
23. Skibola C, Smith M. Potential health impacts of excessive flavonoid intake. *Free Radic Biol Med* 2000; 29: 375–383.
24. Sergediene E *et al*. Pro-oxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure-activity relationships. *FEBS Lett* 1999; 462: 392–396.
25. Cortázar T *et al*. Leishmania panamensis: comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine. *Exp Parasitol* 2007; 116: 475–482.
26. Irene Crespo M *et al*. Differential effects of dietary flavonoids on reactive oxygen and nitrogen species generation and changes in antioxidant enzyme expression induced by proinflammatory cytokines in Chang liver cells. *Food Chem Toxicol* 2008; 46: 1555–1569.
27. Matter W *et al*. The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem Biophys Res Comm* 1992; 186: 624–631.
28. Brusselmans K *et al*. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. *J Biol Chem* 2005; 280: 5636–5645.
29. Casagrande F, Darbon J. Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK1. *Biochem Pharmacol* 2001; 61: 1205–1215.
30. Moridani MY *et al*. Comparative quantitative structure toxicity relationships for flavonoids evaluated in isolated rat hepatocytes and HeLa tumor cells. *Chem Biol Interact* 2002; 139: 251–264.
31. Matsuo M *et al*. Cytotoxicity of flavonoids toward cultured normal human cells. *Biol Pharm Bull* 2005; 28: 253–259.
32. Plochmann K *et al*. Structure–activity relationships of flavonoid-induced cytotoxicity on human leukemia cells. *Arch Biochem Biophys* 2007; 460: 1–9.
33. Schröder-van der Elst JP *et al*. Flavonoids cross the placenta in the rat and are found in fetal brain. *Am J Physiol* 1998; 274: E253–E256.
34. Jang JY *et al*. Antiteratogenic effects of alpha-naphthoflavone on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposed mice in utero. *Reprod Toxicol* 2007; 24: 303–309.
35. Chu KO *et al*. Pharmacokinetic studies of green tea catechins in maternal plasma and fetuses in rats. *J Pharm Sci* 2006; 95: 1372–1381.
36. Waalkens-Berendsen DH *et al*. Embryotoxicity and teratogenicity study with neohesperidin dihydrochalcone in rats. *Regul Toxicol Pharmacol* 2004; 40: 74–79.
37. Chan WH. Impact of genistein on maturation of mouse oocytes, fertilization, and fetal development. *Reprod Toxicol* 2009; 28: 52–58.
38. Richter DU *et al*. Effects of phytoestrogens genistein and daidzein on progesterone and estrogen (estradiol) production of human term trophoblast cells in vitro. *Gynecol Endocrinol* 2009; 25: 32–38.
39. Barrach H *et al*. The applicability of the *in vitro* systems for the evaluation of the significance of pharmacokinetic parameters for the induction of an embryotoxic effect. In: Neubert D *et al*., eds. *Role of Pharmakokinetics in Prenatal and Perinatal Exposure, 3rd Symposium on Prenatal Development*. Germany: George Thieme Publishers, 1978: 323–336.
40. Felgines C *et al*. Bioavailability of flavonone naringenin and its glycosides in rats. *J Am Physiol* 2000; 279: G1148–G1154.
41. Russo A *et al*. Bioflavonoids as antiradicals, antioxidants and DNA clevage protectors. *Cell Biol Toxicol* 2000; 16: 91–98.
42. Hollman PC, Katan MB. Bioavailability and health effects of dietary flavonols in men. *Arch Toxicol Suppl* 1998; 20: 237–248.
43. Conquer JA *et al*. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. *J Nutr* 1998; 128: 593–597.
44. Willhite C. Teratogenic potential of quercetin in the rat. *Food Chem Toxicol* 1982; 20: 75–79.

45. Erlund I *et al*. Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. *Eur J Clin Nutr* 2002; 56: 891–898.
46. Cederberg J *et al*. Combined treatment with vitamin E and vitamin C decreases oxidative stress and improves fetal outcome in experimental diabetic pregnancy. *Pediatr Res* 2001; 49: 755–762.
47. Collins M *et al*. Amelioration of the embryotoxicity of hydroxyurea in whole embryo culture with antioxidant substances. *Teratology* 1991; 43: 417.
48. Herken R. The influence of deoxycytidine monophosphate (dCMP) on the cytotoxicity of hydroxyurea in the embryonic spinal cord of the mouse. *Teratology* 1984; 30: 83–90.
49. DeSesso JM, Goeringer G. The nature of embryoprotective interaction of propylgallate with hydroxyurea. *Reprod Toxicol* 1990; 4: 145–152.
50. DeSesso JM, Goeringer G. Ethoxyquin and nordhydroguaiaretic acid reduce hydroxyurea developmental toxicity. *Reprod Toxicol* 1990; 4: 160–167.
51. DeSesso JM *et al*. Hydroxylamine moiety of developmental toxicants is associated with early cell death: a structure-activity analysis. *Teratology* 2000; 62: 346–355.
52. Pérez-Pastén R *et al*. Effects of dimethylsulphoxide on mice arsenite-induced dysmorphogenesis in embryo culture and cytotoxicity in embryo cells. *Toxicol Lett* 2006; 161: 167–173.
53. Keysar SB *et al*. Hypoxia/reoxygenation-induced mutations in mammalian cells detected by the flow cytometry mutation assay and characterized by mutant spectrum. *Radiat Res* 2010; 173: 21–26.
54. Okada Y *et al*. Hydroxyl radical scavenging action of capsaicin. *Redox Rep* 2002; 7: 153–157.
55. Heo H *et al*. Effect of antioxidant flavanone, naringenin, from Citrus junoson neuroprotection. *J Agric Food Chem* 2004; 52: 1520–1525.
56. Boots A *et al*. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol* 2008; 585: 325–337.
57. Di Giorgio A *et al*. Dimethyl sulfoxide provides neuroprotection in a traumatic brain injury model. *Restor Neurol Neurosci* 2008; 26: 501–517.